ASSEMBLY OF SINGLE-CHAIN ANTIBODY GENES FOR SEMISYNTHETIC LIBRARY v. 2.1

```
                    /     VH gene        / CDR H3 /           JH4b gene           /                G4S linker                /
   EcoRI /  SfiI       Glu       Arg                 6x-12x  TrpGlyGluGlyThrLeuValThrValSerGlyGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySer (SEQ ID NO: 2325)
CCGGAATTCGGCCCAGCCGGCCNNN------NGA (SEQ ID NO: 2322)  NNB---NNBTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCC (SEQ ID NO: 2326)
GGCCTTAAGCCGGGTCGGCCGGNNN------N                    CTNNV---NNVACCCCGGTCCCTTGGGACCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACCGCCTA
          3' end for 40 VH genes                                                                                                                  BamHI
CCGGAATTCGGCCCAGCCGGCCNNN------NAA (SEQ ID NO: 2323) NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N                    TTNNM---NNM
          3' end for 7 VH genes: DP31, DP33, DP39, DP40, flp1, DP47 and DP49                                                  BOLD = IN DGI COLLECTION

CCGGAATTCGGCCCAGCCGGCCNNN------NCA (SEQ ID NO: 2324) NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N                   GTNNM---NNM
          3' end for 4 VH genes: DP2, DP3, DP5 and DP38


All kappa genes:          /          JK1 gene               /  NotI /  PCR primer site    /
  Asp         Pro         TrpThrPheGlyGlnGlyThrLysValGluIleLys (SEQ ID NO: 2327)
  NNN---------CCT         TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG (SEQ ID NO: 2328)
GGNNN---------G         GAACCTGCAAGCCGGTTCCCTGGTTCCACCTTTAGTTTCGCCGGCGTCACACTCAGGTTTTCTAAAGC (SEQ ID NO: 2329)
 (except VK L20)
                          /          JK2 gene               /
                          TyrThrPheGlyGlnGlyThrLysLeuGluIleLys (SEQ ID NO: 2330)
                          TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA (SEQ ID NO: 2331)continued as for JK1
                        GAATGTGAAAACCGGTCCCCTGGTTCGACCTCTAGTTT (SEQ ID NO: 2332)

                          /          JK3 gene               /
                          PheThrPheGlyProGlyThrLysValAspIleLys (SEQ ID NO: 2333)
                          TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA (SEQ ID NO: 2334)continued as for JK1
                        GAAAGTGAAAGCCGGGACCCTGGTTTCACCTATAGTTT (SEQ ID NO: 2335)

                          /          JK4 gene               /
                          LeuThrPheGlyGlyGlyThrLysValGluIleLys (SEQ ID NO: 2336)
                          CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA (SEQ ID NO: 2337)continued as for JK1
                        GAGAGTGAAAGCCGCCTCCCTGGTTCCACCTCTAGTTT (SEQ ID NO: 2338)

                     DPL16 (=v3s1)+
Lambda 3 genes:     /    +v318    /       /  JL2/3 for DPL16+v3s1+v318       /  NotI /  PCR primer site    /
                    Ser         His        ValValPheGlyGlyGlyThrLysLeuThrValLeu (SEQ ID NO: 2339)
                    NNN---------CAT        GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG (SEQ ID NO: 2340)
                  GGNNN---------G        TACACCATAAGCCGCCTCCCTGGTTCGACTGGCAGGATCGCCGGCGTCACACTCAGGTTTTCTAAAGC (SEQ ID NO: 2341)

Lambda 3 gene:      /DPL23 (=VL3.1)/     for DPL23+VL3.1
                    Ser         Ala        GTG---   continued as for JL2/3
                    NNN---------GCA      GTCAC---
                  GGNNN---------C

Lambda 1 genes:     / DPL2+DPL3   /
                    Gln         Gly      for DPL2+DPL3
                    NNN---------GGT        GTG---   continued as for JL2/3
                  GGNNN---------C        CACAC---

Lambda 2 gene:      /    DPL11    /
                    Gln         Leu      for DPL11
                    NNN---------CTC        GTG---   continued as for JL2/3

                  GGNNN---------G        AGCAC---
```

FIGURE 33

ASSEMBLY OF SINGLE-CHAIN ANTIBODY GENES FOR SEMISYNTHETIC LIBRARY v. 2.1

```
                    /     VH gene        / CDR H3 /            JH4b gene           /                 G4S linker                  /
   EcoRI /  SfiI      Glu      Arg                    6x-12x  TrpGlyGluGlyThrLeuValThrValSerSerGlyGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySer (SEQ ID NO: 2325)
CCGGAATTCGGCCCAGCCGGCCNNN------NGA (SEQ ID NO: 2322)  NNB---NNBTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCC (SEQ ID NO: 2326)
GGCCTTAAGCCGGGTCGGCCGGNNN------N                      CTNNV---NNVACCCCGGTCCCTTGGGACCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACCGCCTA
          3' end for 40 VH genes                                                                                                                  BamHI
CCGGAATTCGGCCCAGCCGGCCNNN------NAA (SEQ ID NO: 2323) NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N                      TTNNM---NNM
          3' end for 7 VH genes: DP31, DP33, DP39, DP40, flp1, DP47 and DP49                                                  BOLD = IN DGI COLLECTION

CCGGAATTCGGCCCAGCCGGCCNNN------NCA (SEQ ID NO: 2324) NNK---NNK    continued as for J4b gene and linker
GGCCTTAAGCCGGGTCGGCCGGNNN------N                      GTNNM---NNM
          3' end for 4 VH genes: DP2, DP3, DP5 and DP38


All kappa genes:          /          JK1 gene               /  NotI /  PCR primer site    /
  Asp         Pro         TrpThrPheGlyGlnGlyThrLysValGluIleLys (SEQ ID NO: 2327)
  NNN---------CCT         TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG (SEQ ID NO: 2328)
GGNNN---------G         GAACCTGCAAGCCGGTTCCCTGGTTCCACCTTTAGTTTCGCCGGCGTCACACTCAGGTTTTCTAAAGC (SEQ ID NO: 2329)
 (except VK L20)
                          /          JK2 gene               /
                          TyrThrPheGlyGlnGlyThrLysLeuGluIleLys (SEQ ID NO: 2330)
                          TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA (SEQ ID NO: 2331)continued as for JK1
                         GAATGTGAAAACCGGTCCCCTGGTTCGACCTCTAGTTT (SEQ ID NO: 2332)

                          /          JK3 gene               /
                          PheThrPheGlyProGlyThrLysValAspIleLys (SEQ ID NO: 2333)
                          TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA (SEQ ID NO: 2334)continued as for JK1
                         GAAAGTGAAAGCCGGGACCCTGGTTTCACCTATAGTTT (SEQ ID NO: 2335)

                          /          JK4 gene               /
                          LeuThrPheGlyGlyGlyThrLysValGluIleLys (SEQ ID NO: 2336)
                          CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA (SEQ ID NO: 2337)continued as for JK1
                         GAGAGTGAAAGCCGCCTCCCTGGTTCCACCTCTAGTTT (SEQ ID NO: 2338)

                    DPL16 (=v3s1)+
Lambda 3 genes:     /    +v318    /       /  JL2/3 for DPL16+v3s1+v318       /  NotI /  PCR primer site    /
                    Ser         His        ValValPheGlyGlyGlyThrLysLeuThrValLeu (SEQ ID NO: 2339)
                    NNN---------CAT        GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG (SEQ ID NO: 2340)
                   GGNNN---------G        TACACCATAAGCCGCCTCCCTGGTTCGACTGGCAGGATCGCCGGCGTCACACTCAGGTTTTCTAAAGC (SEQ ID NO: 2341)

Lambda 3 gene:      /DPL23 (=VL3.1)/     for DPL23+VL3.1
                    Ser         Ala        GTG---   continued as for JL2/3
                    NNN---------GCA       GTCAC---
                   GGNNN---------C

Lambda 1 genes:     / DPL2+DPL3   /
                    Gln         Gly       for DPL2+DPL3
                    NNN---------GGT        GTG---   continued as for JL2/3
                   GGNNN---------C         CACAC---

Lambda 2 gene:      /    DPL11    /
                    Gln         Leu       for DPL11
                    NNN---------CTC        GTG---   continued as for JL2/3

                   GGNNN---------G         AGCAC---
```

FIGURE 33

IGF-5 (SEQ ID NOS 2193 (DNA) and 2194-2199 (protein), respectively, in order of appearance)

```
GACTACAAAG ACTCGTGGTT GAATTTTCGG TATGTTGCTG GGCGTGCTTA GGTTTCAGAT TCTTCTGTGG CCGTTTCCTA AGGATTGTGT TCAGATGAAA GATATTTTTT ATTCGTTGTT GGCTAGTTTG GCGGCCGCA  139
D Y K D  S W L  N F R  Y V A G  R A Q  V S D  S S V A  V S .  G L C  S D E R  Y F L  F V V  G Q F G  G R
 T T K  T R G .  I F G  H L L  G V L R  F Q I  L L W  P F P K  D C V  Q M K  D I F Y  S L L  A S L  A A A
  L Q R  L V V  E F S V  C C W  A C L  G F R F  F C G  R F L  R I V F  R . K  I F F  I R C W  L V W  R P
```

IGF-8 (SEQ ID NOS 2200 (DNA) and 2201-2205 protein, respectively, in order of appearance)

```
GACTACAAAG ACGCGGTTGC GGCTGCTGTT GCTCCTTGGG GGTGATGAGC CTTTTTATGG GCTTCTCCGT ATGCTGATTG GTCGTGGGTC TGCGGCCGCA  100
D Y K D  A V A  A A V  A P W G  .  .  A  F L W  A S P Y  A D W  S W V  C G R
 T T K  T R L R  L L L  L L G  G D E P  F Y G  L L R  H L I G  R G S  A A A
  L Q R  R G C  G C C C  S L G  V M S  L F H G  F S V  C .  L V V G L  R P
```

IGF-G5 (SEQ ID NOS 2206 (DNA) and 2207-2210 (protein), respectively, in order of appearance)

```
GACTACAAAG ACTGGTTGGT TTGCTTGGGT GTGATGATTA GCTTTTTCTG TTTAGGGGGT CGGTGTGGCT TTTTACTCAG CGTTGGCTGC CTTGTTGTGT GCCCATAGTG CTTCTTTGGT GTGTGGTGCG GCGGCCGCA 139
D Y K D  W L V  C L G  V M I S  F F C  L G G  R C G F  L L S  V G C  L V V C  P Q C  F F G  V W C G  G R
 T T K  T G W F  A W V  .  .  L  A F S V  Q G V  G V A  F Y S A  L A A  L L C  A H S A  S L V  C G A  A A A
  L Q R  L V G  L L G C  D D Q  L F L  F R G S  V W L  F T Q  R W L P  C C V  P I V  L L W C  V V R  R P
```

IGF-7 (SEQ ID NOs 2211 (DNA) and 2212-2217 (protein), respectively, in order of appearance)

```
GACTACAAAG ACCCGGATTG GGTGTTGTAG CTGATTAGTT TGGGGTTGGA GGGGATGTAG ATTGGCTGAT GGGTTTTATG CGTTTTTGAT GGCGCTGGCT GGGGCGGCCG CA 112
D Y K D  P D W  V L Q  L I S L  G L E  G M Q  I G .  W  V L C  V F D  G A G W  G G R
 T T K  T R I G  C C S  . L V  W G W R  G C R  L A D  G F Y A  F L M  A L A  G A A A
  L Q R  P G L  G V V A  D Q F  G V G  G D V D  W L M  G F M  R F .  W  R W L  G R P
```

FIGURE 19

IGF-5 (SEQ ID NOS 2193 (DNA) and 2194-2199 (protein), respectively, in order of appearance)

```
GACTACAAAG ACTCGTGGTT GAATTTTCGG TATGTTGCTG GGCGTGCTTA GGTTTCAGAT TCTTCTGTGG CCGTTTCCTA AGGATTGTGT TCAGATGAAA GATATTTTTT ATTCGTTGTT GGCTAGTTTG GCGGCCGCA  139
D Y K D  S W L  N F R  Y V A G  R A Q  V S D  S S V A  V S .  G L C  S D E R  Y F L  F V V  G Q F G  G R
 T T K  T R G .  I F G  H L L  G V L R  F Q I  L L W  P F P K  D C V  Q M K  D I F Y  S L L  A S L  A A A
  L Q R  L V V  E F S V  C C W  A C L  G F R F  F C G  R F L  R I V F  R . K  I F F  I R C W  L V W  R P
```

IGF-8 (SEQ ID NOS 2200 (DNA) and 2201-2205 protein, respectively, in order of appearance)

```
GACTACAAAG ACGCGGTTGC GGCTGCTGTT GCTCCTTGGG GGTGATGAGC CTTTTTATGG GCTTCTCCGT ATGCTGATTG GTCGTGGGTC TGCGGCCGCA  100
D Y K D  A V A  A A V  A P W G  .  .  A  F L W  A S P Y  A D W  S W V  C G R
 T T K  T R L R  L L L  L L G  G D E P  F Y G  L L R  H L I G  R G S  A A A
  L Q R  R G C  G C C C  S L G  V M S  L F H G  F S V  C .  L  V V G L  R P
```

IGF-G5 (SEQ ID NOS 2206 (DNA) and 2207-2210 (protein), respectively, in order of appearance)

```
GACTACAAAG ACTGGTTGGT TTGCTTGGGT GTGATGATTA GCTTTTTCTG TTTAGGGGGT CGGTGTGGCT TTTTACTCAG CGTTGGCTGC CTTGTTGTGT GCCCATAGTG CTTCTTTGGT GTGTGGTGCG GCGGCCGCA 139
D Y K D  W L V  C L G  V M I S  F F C  L G G  R C G F  L L S  V G C  L V V C  P Q C  F F G  V W C G  G R
 T T K  T G W F  A W V  .  .  L  A F S V  Q G V  G V A  F Y S A  L A A  L L C  A H S A  S L V  C G A  A A A
  L Q R  L V G  L L G C  D D Q  L F L  F R G S  V W L  F T Q  R W L P  C C V  P I V  L L W C  V V R  R P
```

IGF-7 (SEQ ID NOs 2211 (DNA) and 2212-2217 (protein), respectively, in order of appearance)

```
GACTACAAAG ACCCGGATTG GGTGTTGTAG CTGATTAGTT TGGGGTTGGA GGGGATGTAG ATTGGCTGAT GGGTTTTATG CGTTTTTGAT GGCGCTGGCT GGGGCGGCCG CA 112
D Y K D  P D W  V L Q  L I S L  G L E  G M Q  I G .  W  V L C  V F D  G A G W  G G R
 T T K  T R I G  C C S  .  L V  W G W R  G C R  L A D  G F Y A  F L M  A L A  G A A A
  L Q R  P G L  G V V A  D Q F  G V G  G D V D  W L M  G F M  R F .  W  R W L  G R P
```

FIGURE 19